



(RESEARCH ARTICLE)



## Antibacterial test of ethanol extract of "Cocor-Bebek" leaves (*Kalanchoe pinnata*) against the growth of *Staphylococcus aureus* and *Salmonella typhi*

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### Abstract

This study aims to determine the antibacterial effect of the Ethanol Extract of Cocor bebek leaves (*Kalanchoe pinnata*) on the growth of *Staphylococcus aureus* and *Salmonella typhi*. The study is a true experimental, using a posttest only control group design with the independent variable being the extract of cocor bebek leaves (*Kalanchoe pinnata*). This study found that extracts of cocor bebek leaves (*Kalanchoe pinnata*) with a concentration of 7.5% and 15% had no inhibitory power, while at concentrations of 30% and 60% had weak inhibitory power as the minimum inhibitory power, and a concentration of 100% had inhibitory power, and strong on the growth of *Staphylococcus aureus* bacteria. Meanwhile for the growth of *Salmonella typhi*, a concentration of 7.5% from 3 repetitions had an average inhibition zone of 1.2 mm. for a concentration of 15% has an average inhibition zone diameter of 3 repetitions of 2.2 mm. concentration of 30% from 3 rolls has an average inhibition zone of 12.6 mm. for a concentration of 60% has an average inhibition zone of 3 repetitions of 19.8mm. There is antibacterial activity of ethanolic extract of cocor bebek leaf (*Kalanchoe pinnata*) against the growth of *Staphylococcus aureus* and *Salmonella typhi*.

**Keywords:** Antibacterial Agents; Ethanol; *Salmonella Typhi*; *Staphylococcus Aureus*

### 1. Introduction

*Staphylococcus aureus* is a gram-positive facultative anaerobic bacterium that acts as a commensal organism and an important opportunistic pathogen for humans, which can cause bacteremic sepsis, endocarditis, pneumonia, osteomyelitis, arthritis, and skin diseases [1–4]. A study in Dr. Soeradji Tirtonegoro's hospital revealed that isolates were resistant to tetracycline antibiotics (64.8%), erythromycin (53.7%), and cloxacillin (40.7%) [5]. According to the Indonesian Ministry of Health, various studies have found that around 40–62% of antibiotics are misused, among others, for diseases that do not require antibiotics [6]. Antimicrobial resistance increases worldwide, resulting in more difficult infections to treat and higher mortality, morbidity, and costs [7]. In the research, Sandika & Suwandi [8] explained an increase in antibiotic resistance to *Salmonella typhi* bacteria because the bacteria can be resistant to antibiotics.

The bacterium *Salmonella typhi* is a cause of typhoid fever and has high mortality and morbidity worldwide [9]. Global problems related to typhoid fever are estimated to reach 12 million cases and 130,000 deaths in 2010 due to typhoid fever [10]. The prevalence of typhoid fever in Indonesia is 1.60%, the highest occurring in the 5–14 year age group because children pay less attention to personal hygiene and snacking habits that can cause typhoid fever transmission [11].

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Cocor-Bebek leaves are one of the biological diversity that has the potential to be developed as traditional medicine [12,13]. Cocor bebek is an ornamental plant that is very easy to cultivate and use in traditional medicine because it has antipyretic, anti-inflammatory, and antimicrobial properties. The main chemical elements that have antimicrobial activity in cocor duck leaves are flavonoid compounds, tannins, cinnamic acid, and bufadienolides [2,14].

The cocor bebek plant contains flavonoids. It may inhibit cell membrane function by forming complex compounds consisting of extracellular and dissolved proteins to damage bacterial cell membranes and is followed by intracellular compounds and inhibit cell membrane function disrupting cell membrane permeability and inhibiting enzymes, such as ATPase and phospholipase. In addition to flavonoids, there are saponins whose mechanism of action causes the leakage of proteins and enzymes from the cell. Saponins can reduce the surface tension of bacterial cell walls and damage membrane permeability [3,4,15,16].

This research was carried out with the aim of knowing Antibacterial Test of Ethanol Extract of "Cocor-Bebek" Leaves (*Kalanchoe pinnata*) Against the Growth of *Staphylococcus aureus* and *Salmonella typhi*.

## 2. Methods

This experimental study was carried out at the Biomedical Laboratory of Halu Oleo University Medical College using a post-test-only control design. The independent variable is the treatment of cocor bebek leaves (*Kalanchoe pinnata*) extract with different concentrations from ethanol extract. The antibiotics Ciprofloxacin and Ceftriaxone were positive control and distilled water. As a negative control against *Staphylococcus aureus* and *Salmonella typhi* cultures with three repetitions with different extract concentrations.

Samples of cocor bebek leaves were first cleaned and then cut into small pieces of  $\pm 2$  cm. Next, the samples were collected in one container. Then, it was dried in an oven at 40°C for four days, and a dry sample was obtained. Extraction was carried out by the maceration method. Samples of cocor bebek leaves (*Kalanchoe pinnata*) were macerated with ethyl acetate solvent with a ratio of 10 parts of the sample to the filtered solvent in 75 parts of the solvent or until the sample was submerged. The maceration vessel was tightly closed and left for three days while stirring and stored in a place not exposed to sunlight. After maceration, the results were then evaporated using a Rotator vacuum evaporator to obtain a thick ethanol extract of cocor bebek leaves (*Kalanchoe pinnata*).

## 3. Results

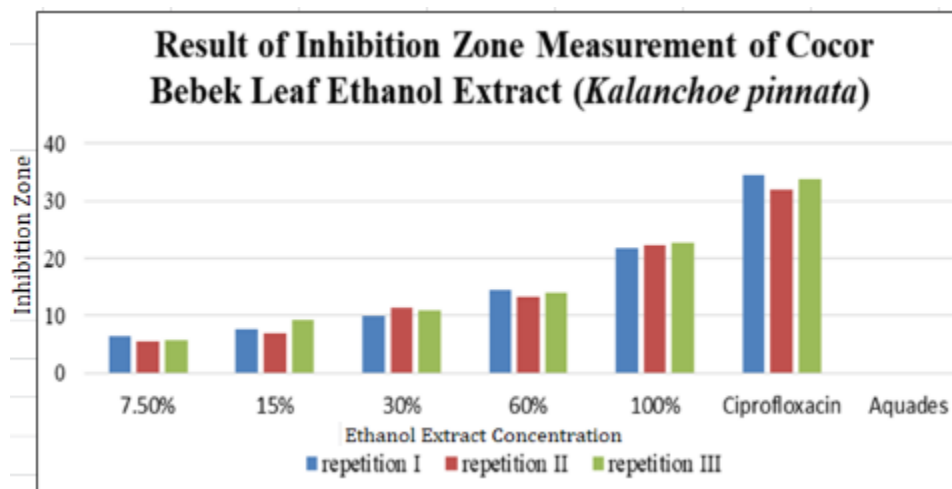
Based on the data in Table 1 shows, the results of processing samples of cocor bebek leaves (*Kalanchoe pinnata*), both wet samples (4.8 kg) and dry samples (547.94 grams), so a total of 27.54 grams of extract weight was obtained. The extraction process is carried out using a commonly used method, maceration, with the working principle of diffusion, where the solvent will enter the material cell. Dry samples of cocor bebek leaves were macerated with 96% ethanol for 3x24 hours. The amount of solvent used is 1:3 with the sample. The filtrate was collected and evaporated using a rotary vacuum evaporator at a temperature of 60°C to obtain a thick extract of 27.54 g.

**Table 1** Extraction results of cocor bebek leaves (*Kalanchoe pinnata*) using ethanol

Wet sample	dry sample	Extract weight
4.8 kg	547.93 g	27.54 g

The extraction process is carried out by maceration, through the working principle of diffusion, where the solvent will enter the material cell. Dry samples of cocor bebek leaves were macerated with 96% ethanol for 3x24 hours at room temperature. The amount of solvent used is 1:3 with the sample. The filtrate was collected and evaporated with a rotary vacuum evaporator at a temperature of 60 C to obtain a thick extract of 27.54 g.

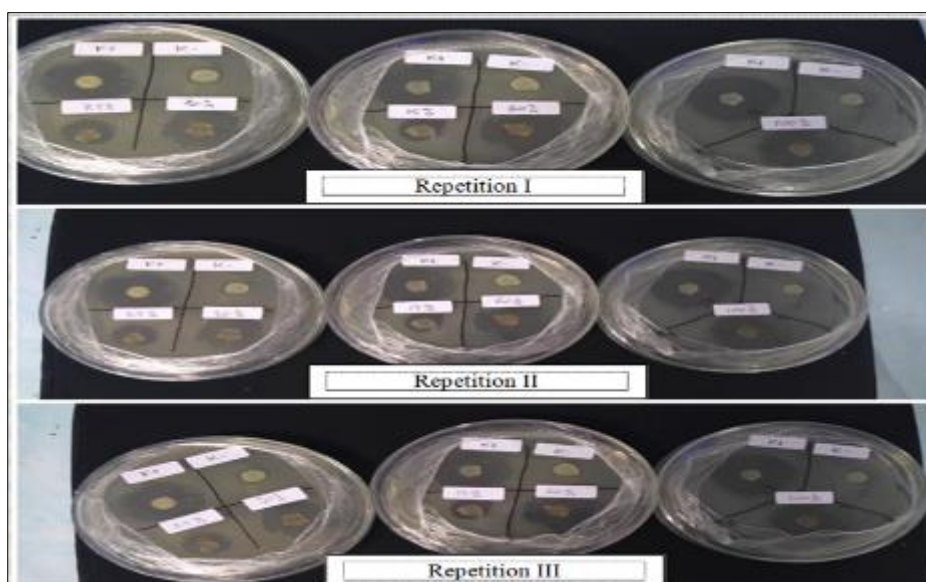
Based on the interpretation in Figure 1, the interpretation of the strength of the inhibition zone, each concentration of cocor bebek leaves (*Kalanchoe pinnata*) extract has antibacterial activity at a particular concentration with weak inhibitory zone ability, so it is strong against the growth of *Staphylococcus aureus* bacteria. Ciprofloxacin positive control was sensitive to *Staphylococcus aureus* bacteria and had a relatively broad zone of inhibition.



**Figure 1** Inhibition Zone Measurement Results for Ethanol Extract of Cocor Bebek Leaves (*Kalanchoe pinnata*) against *Staphylococcus aureus* bacteria

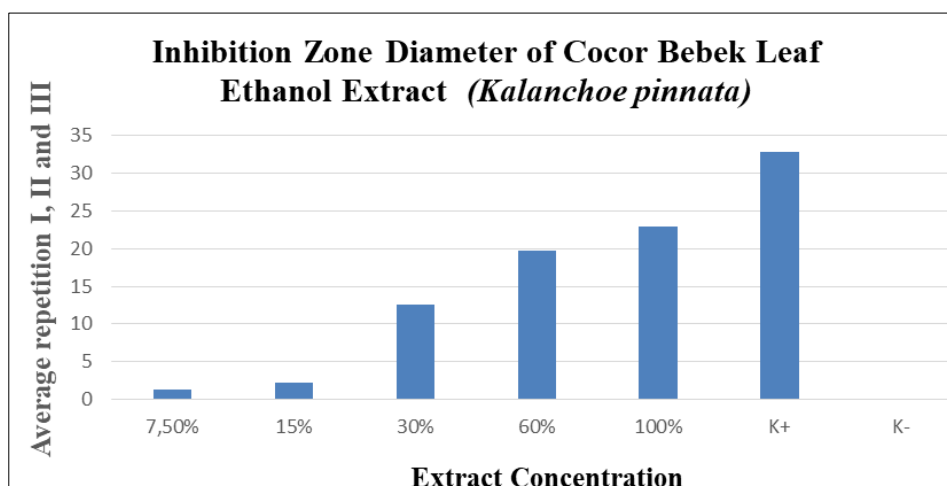
**Table 2** Interpretation of Measuring Results of Average Inhibitory Zone Diameter against *Staphylococcus aureus*

Ethanol Extract Concentration	Inhibition Zone Diameter (mm)			Average (mm)	Interpretation
	I	II	III		
7.5 %	6.5	5.5	5.75	5.91	None
15 %	7.5	7	9.25	7.91	None
30 %	10	11.5	11	10.83	Weak
60 %	14.5	13.25	14	13.91	Weak
100 %	21.75	22.25	22.75	22.25	Strong
Positive Control	34.5	32	33.75	33.41	Strong
Negative Control	0	0	0	0	None



**Figure 2** The results of the antibacterial activity of cocor bebek leaves (*Kalanchoepinnata*)

Based on the data in Table 2, it was found that the extract of cocor bebek leaves (*Kalanchoe pinnata*) with a concentration of 7.5% and 15% had no inhibitory power. In comparison, concentrations of 30% and 60% had weak inhibition as the minimum inhibitory power, and a concentration of 100% had potent inhibition on the growth of *Staphylococcus aureus* bacteria. Positive control Ciprofloxacin was sensitive to *Staphylococcus aureus* and had a relatively broad zone of inhibition.



**Figure 3** Average Diameter of the Inhibitory Zone on the Growth of *Salmonella typhi* bacteria

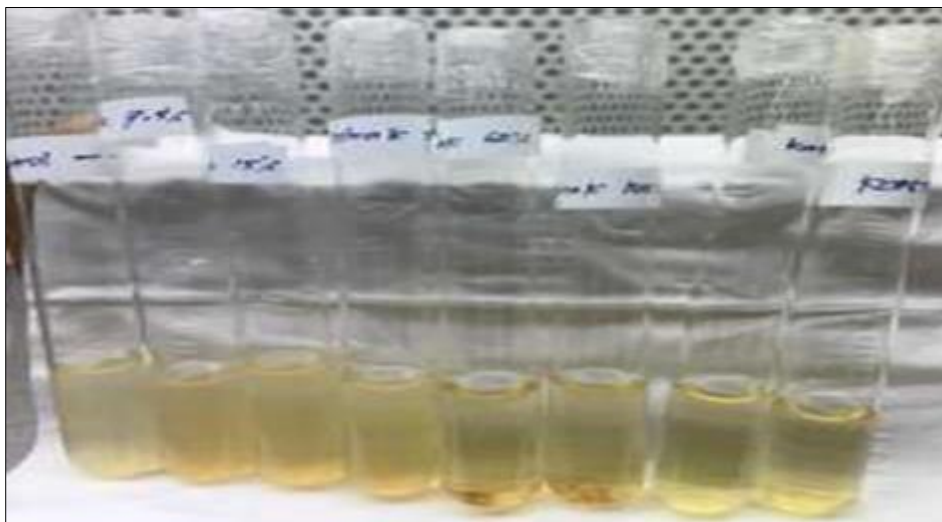
**Table 3** Results of Measurement of Inhibitory Zone Diameter from Cocor bebek Leaves Extract (*Kalanchoe pinnata*) Against the Growth of *Salmonella typhi* Bacteria

Ethanol Extract Concentration	Repetition (mm)			Average diameter of inhibition zone (mm)	Interpretation
	I	II	III		
100%	19.7	24.2	26.7	23	Strong
60%	17	20.7	21.7	19.8	Moderate
30%	10.2	17.2	10.7	12.6	Weak
15%	2.2	2	2.4	2.2	None
7.50%	1	1.8	1.3	1.3	None
Control +	31.2	31.5	36.2	32.9	Strong
Control -	0	0	0	0	None

Table 3 and Figure 3 show that various ethanol extracts of cocor bebek leaves (*Kalanchoe pinnata*) were inhibited in their respective circumferences of the disc. The concentration of 7.5% from 3 repetitions had an average inhibition zone of 1.2 mm. The 15% and 30% concentration has an average inhibition zone diameter of 3 repetitions of 2.2 mm and 12.6 mm. A concentration of 60% has an average inhibition zone of 3 repetitions of 19.8 mm. concentration of 100% from 3 repetitions had an average inhibition zone of 23 mm. while for the control (+) the antibiotic ceftriaxone used, the average diameter of the inhibition zone from 3 repetitions was 32.9 mm. At the same time, distilled water is a control (-) which obtained the average diameter of the inhibition zone from 3 repetitions of 0 mm.

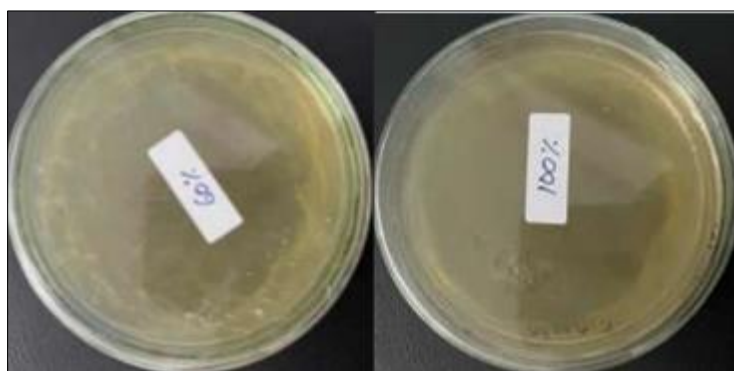
Measurement of the minimum inhibitory concentration was carried out by the liquid dilution method. This test was carried out by looking at the clarity in the test tube, mixed with cocor bebek leaf ethanol extract in 100%, 60%, 30%, 15%, and 7.5%. The test tubes were filled with 5 ml of liquid medium (MHB) each, and 20µl of cocor bebek leaf ethanol extract was added, and then 20µl of bacterial suspension was planted. Changes in color or clarity in the test tube after being incubated for 24 hours at 37°C were observed.

Figure 4 shows that the concentration of ethanol extract 7.5%, 15%, and 30%, and control (-) there is turbidity while at concentrations of 60%, 100% and control (+) and control (1) there is clarity. Control (-) showed turbidity, meaning no antibacterial activity by aquades against *Salmonella typhi* bacteria. Turbidity in extracts at 7.5%, 15%, and 30% indicated no antibacterial activity by extracts against *Salmonella typhi* bacteria. The clarity that occurred at the extract concentration of 60% and 100% indicated the presence of antibacterial activity by the extract against *Salmonella typhi* bacteria. Likewise, in control (+), clarity indicated the presence of antibacterial activity by the antibiotic ceftriaxone against *Salmonella typhi* bacteria, while control 1 is pure media without any mixture of bacteria or extracts.



**Figure 4** Results of MIC Observation of Ethanol Extract of Cocor bebek Leaves against the Growth of *Salmonella typhi* Bakteri

However, further tests were carried out to strengthen the visual observations of the researchers toward the MIC of ethanolic extract of cocor bebek leaves. It aimed to ensure that the MIC of ethanolic extract of cocor bebek leaves was present at a concentration of 60%. This test was carried out using the respective concentrations of 60% and 100% of the previously used medium-filled test tubes (MHB). Concentrations of 60% and 100% of the previously used test tubes containing media (MHB) were poured as much as 100 l into a petri dish filled with NA media and then stored in an incubator for 1x24 hours reobserved.



**Figure 5** Results of the Advanced KHM Test Results of Ethanol Extract of Cocor bebek Leaves on the growth of *Salmonella typhi* bacteria

Figure 5 shows that at a concentration of 60% cocor bebek leaf ethanol extract, there was a growth of *Salmonella typhi* bacteria. In comparison, at 100% cocor bebek leaf ethanol extract concentration, there was no growth of *Salmonella typhi* bacteria. Therefore, based on the results, we concluded that the Minimum Inhibitory Concentration (MIC) of the ethanolic extract of cocor bebek leaves on the growth of *Salmonella typhi* bacteria was found at a concentration of 100%.

#### 4. Discussion

The sample used in this study was cocor bebek leaves (*Kalanchoe pinnata*) which were then extracted using ethanol and diluted with distilled water. It was divided into five concentrations, namely 7.5%, 15%, 30%, 60%, and 100%. The positive control used was liquid ciprofloxacin which was dripped on 20 L filter paper. The bacteria used were *Staphylococcus aureus* by planting on an MHA medium, and then each concentration was dripped on filter paper on top of the MHA medium.

Extract of cocor bebek leaves (*Kalanchoe pinnata*) was prepared using ethanol as solvent. The basis for choosing ethanol as a solvent is because ethanol can inhibit the work of enzymes to minimize the occurrence of enzymatic reactions (e.g., hydrolysis of flavonoids). Besides that, 70% ethanol can also take the active target components (flavonoids) optimally and selectively in extracting components in simplicia ingredients [17]. Pinilih and Hidayat [18] stated that ethanol is polar, so the extracted compounds are relatively polar. The polarity of these compounds causes these compounds to penetrate bacterial cell walls more easily. The main chemical elements that have antimicrobial activity in cocor bebek leaves are flavonoid compounds, tannins, cinnamic acid, and bufadienolides [5,19].

This study used distilled water as a negative control. Based on research by Rizal [20], it was found that distilled water as negative control did not provide an inhibition zone, which means it has no effect as an antibacterial. This study used ciprofloxacin as a positive control which has a mechanism of action similar to the active compound content of cocor bebek leaves by inhibiting nucleic acid synthesis. Pratiwi [21] stated that this class of antibiotics could enter cells by passive diffusion through water-filled protein channels (porins) on the bacterial outer membrane intracellularly. Uniquely these drugs inhibit bacterial DNA replication. By interfering with DNA gyrase (topoisomerase II) work during bacterial growth and reproduction, this is what causes the positive control to get an inhibition zone on *Staphylococcus aureus* bacteria.

The ethanolic extract of cocor bebek leaves (*Kalanchoe pinnata*) has secondary metabolites, including flavonoids. Santoso et al, [22] stated that flavonoids are a group of phenolic phytochemicals that function as antimicrobials where these compounds will interfere with energy metabolism. In addition, flavonoids can damage cell membranes that bind to extracellular and dissolved proteins which will cause cell lysis and the release of intracellular compounds [23].

Flavonoid compounds and tannins are included in phenol compounds. This compound has antibacterial properties by inhibiting the metabolism of bacterial cells. This inhibition causes denaturation of bacterial proteins, inhibits the formation of cytoplasmic proteins and nucleic acids, and inhibits ATP-ase binding to cell membranes. These compounds interfere with and affect the integrity of the cytoplasmic membrane [8,23].

In addition, cinnamic acid compounds have antibacterial activity as antimicrobials by inhibiting microbial protein synthesis. This inhibition of protein synthesis occurs in various ways. One of them, antibacterial binds to the 30S ribosomal component, which causes the mRNA code to be misread by the tRNA during protein synthesis. As a result, abnormal and non-functional proteins will be formed for the bacterial cells in question. At the same time, bufadienolides inhibit the synthesis of bacterial nucleic acids, which bacterial cells generally require [18].

A study on the activity testing of the ethanolic extract of cocor bebek leaves (*Kalanchoe pinnata*) has been carried out on the growth of *Salmonella typhi* bacteria cultured in Petri dishes using NA (Nutrient Agar) medium. This research was conducted using the diffusion and delusional methods by looking at the inhibition zone and the clarity formed in the tested samples. The sample used in this study was cocor bebek leaves (*Kalanchoe pinnata*) extracted using ethanol as a solvent and diluted with distilled water to obtain five concentrations, namely 7.5%, 15%, 30%, 60%, and 100%. The positive control that has been used is ceftriaxone powder which is then diluted using distilled water and dripped onto filter paper as much as 20 µl.

Ceftriaxone antibiotics work by binding to penicillin-binding proteins in bacteria and inhibiting bacterial cell wall synthesis, which causes cell lysis and death. This antibiotic works by inhibiting the synthesis of mucopeptides required to form bacterial cell walls, namely by inhibiting the third stage of the transpeptidase reaction in a series of cell wall formation reactions [24].

Based on the test results, the antibacterial activity of the ethanolic extract of cocor bebek leaves (*Kalanchoe pinnata*) varied according to the concentration. The antibacterial test observation of the ethanolic extract of cocor bebek leaves has potential as an antibacterial with weak, medium, and strong categories, in line with research conducted by Pinilih and Hidayat [18], where concentrations of 90% and 100% were classified into strong growth inhibitory responses. This study obtained an inhibition zone diameter of 23 mm at 100% concentration, classified in the solid inhibitory response



category, and a concentration of 60% of 19.8 mm, which was also included in the category of solid inhibitory response. The concentration of 30% indicated the presence of an inhibition zone with an average diameter of the inhibition zone of 12.6 mm. Even though it has a weak inhibitory response, it should be noted that the active compound in the ethanolic extract of cocor bebek leaves (*Kalanchoe pinnata*) has properties that are in line with the high concentration level and the inhibition response to the growth of the tested bacteria. Meanwhile, at concentrations of 7.5% and 15% of cocor bebek (*Kalanchoe pinnata*) leaf ethanol extract given to *Salmonella typhi* bacteria, there was an inhibitory response even though it was included in the category of no inhibition zone based on Greenwood classification. It is also in line with Sylvia [25] which showed that the 30% concentration of cocor bebek leaves ethanol extract was classified as a weak inhibitory response, while concentrations of 15% and 7.5% were categorized as having no inhibitory response.

The difference in the inhibition zone occurs due to the different levels of secondary metabolites contained in the sample extract [26]. The higher the concentration used, the larger the inhibition zone formed. It was due to the more active compounds present in the extract. Increasing the concentration of the ethanolic extract of cocor bebek leaves (*Kalanchoe pinnata*) affects the diameter of the inhibition zone formed. Different diameters of the inhibition zone indicate the ability of different extract concentrations to inhibit the growth of the tested bacteria [27].

The study results showed that the inhibition zone formed was marked by a clear area around the paper disc or paper disc planted on NA media (Nutrienagar) with antibacterial inhibition proving that cocor bebek leaves (*Kalanchoe pinnata*) extract inhibited the growth of *Salmonella Typhi* bacteria. Most of the potential inhibitory levels of cocor bebek leaf extract are taken, including aggressive chemicals, so that the chemical content of the material that is expected to be bacteriostatic is neutralized. It is supported by a statement stating that the extraction method using ethanol will absorb more active chemicals from the material. At the same time, the active substances that are suspected of having antibacterial power are Cinnamic acid which inhibits the synthesis of microbial protein, flavonoids, alpha-tocopherol, which works by inhibiting the metabolism of microbial cells, and bufadienolide, which works by destroying microbial nucleic acids [16,28].

The ethanolic extract of cocor bebek leaves (*Kalanchoe pinnata*) has secondary metabolites, including flavonoids. Flavonoids can damage cell membranes that bind to extracellular and dissolved proteins which will cause cell lysis and the release of intracellular compounds [23]. Pramuningstiyah [29] stated that flavonoids are a group of phenolic phytochemicals that function as antimicrobials where these compounds will interfere with energy metabolism.

Flavonoid compounds and tannins are included in phenolic compounds with antibacterial properties. It may inhibit bacterial cell metabolism and bacterial protein denaturation. It also inhibits the formation of cytoplasmic proteins, nucleic acids, and ATP-ase bonds in cell membranes. It means these compounds will interfere with and affect cytoplasmic membrane integrity, resulting in leakage of intracellular material and eventually leading to bacterial cell lysis. In addition, cinnamic acid compounds have antibacterial activity as antimicrobials by inhibiting microbial protein synthesis. At the same time, bufadienolides inhibit bacterial nucleic acid synthesis [13,18].

Measurement of the minimum inhibitory concentration (MIC) obtained clarity began to appear at a concentration of 60% cocor bebek leaves (*Kalanchoe pinnata*) ethanol extract through visual observation. However, after further MIC test, clarity was obtained at 100% cocor bebek leaf ethanol extract concentration. It explains that the ethanolic extract of cocor bebek leaves (*Kalanchoe pinnata*) can inhibit bacterial growth, but in this study, it has not been confirmed if it can kill bacteria.

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## 5. Conclusion

The ethanolic extract of cocor bebek leaves (*Kalanchoe pinnata*) has antibacterial activity against *Staphylococcus aureus* cultures. The minimal inhibition level of the ethanolic extract of cocor bebek leaves (*Kalanchoe pinnata*) on the growth of *Staphylococcus aureus* was 30%.

There is antibacterial activity of cocor bebek (*Kalanchoe pinnata*) leaf ethanol extract against the growth of *Salmonella typhi* bacteria. There is an inhibitory zone of cocor bebek leaves (*Kalanchoe pinnata*) ethanol extract at 30% weak concentration, 60% moderate concentration, and 100% strong concentration against the growth of *Salmonella typhi* bacteria. The formation of a minimum inhibitory concentration (MIC) of the ethanolic extract of the leaves of cocor bebek (*Kalanchoe pinnata*) against *Salmonella typhi* bacteria is at a concentration of 100%.

### Suggestion

This study uses a limited amount of extract concentration. It cannot be determined at what concentration the optimum effect as an alternative antibacterial drug, so this research can be used as a scientific reference or reference for

researchers who carry out further experimental research. For further researchers, it is better to increase the amount of extract concentration to determine the optimal concentration to inhibit the growth of the test bacteria.

Further research is needed to look at the Minimum Kill Concentration (KBM). This research can be used as a reference for the scientific development of cocor bebek leaves as raw materials for natural medicine. It can be used as a source of information regarding the use of cocor bebek leaves in the health sector. The next researcher is expected to measure the inhibition zone by drawing a line right in the middle of the disc.

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## Compliance with ethical standards

### *Acknowledgments*

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### *Disclosure of conflict of interest*

There is no conflict of interest for the author.

### *Ethical considerations*

This study obtained ethical feasibility under the Health Research Ethics Committee of the College of Medicine, Halu Oleo University.

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